Hemicellulases of White- and Brown-rot Fungi in Relation to Host Preferences

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Contents

1. Introduction

Brown- and white-rot fungi depend upon various polymeric carbohydrates in wood cell walls to furnish their metabolic needs. Hemicelluloses are one of the principal cell wall constituents of wood and are an important carbon source for the wood-decaying fungi (E. B. Cowling, 1961; T. K. Kirk and T. L. Highley, 1973). The wood-decay fungi depolymerize the hemicelluloses into metabolizable products via enzymes (hemicellulases and glycosidases). Hemicellulose-degrading enzymes are obviously important to wood decay. Except for pentosanases, however, very few studies have been made of these enzymes from wood-decay fungi.

The mono- and polysaccharides in the environment in which a fungus is growing can affect quantitatively and qualitatively the spectrum of polysaccharide-degrading enzymes secreted by the fungus (P. Albersheim and H. J. Anderson-Prouty, 1975). Thus the relative activities of these enzymes may provide the proper environment for rapid fungal growth in susceptible hosts. The objective of the present study was to compare the composition of induced extracellular hemicellulose-de-

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grading enzymes of a white- and brown-rot fungus to determine if differences might explain their respective preference for hardwoods and softwoods.

2. Materials and Methods

The brown-rot fungus *Poria placenta* (Fr.) Cke. (Madison 698) and the white-rot fungus *Coriolus versicolor* (L. ex Fr.) (Madison 697) were used here because of the considerable information already available about their degradative activities in wood (E. B. Cowling, 1961; T. K. Kirk and T. L. Highley, 1973).

The fungi were grown in stationary cultures on a liquid medium with a carbon source and a basal salts solution in distilled water containing (per l.):

NH_4NO_3 (2.0 g)	$MnCl_2 \cdot 4\ H_2O$ (0.036 mg)
KH_2PO_4 (2.0 g)	$ZnSO_4 \cdot 7\ H_2O$ (0.31 mg)
$Mg \cdot SO_4 \cdot 7\ H_2O$ (0.5 g)	$CuSO_4 \cdot 5\ H_2O$ (0.039 mg)
$CaCl_2 \cdot 2\ H_2O$ (0.1 g)	$(NH_4)\ 6\ Mo_7O_{24} \cdot 4\ H_2O$ (0.018 mg)
thiamine hydrochloride (1.0 mg)	$FeSO_4 \cdot 7\ H_2O$ (0.015 mg)

Culture vessels were 250 ml Erlenmeyer flasks containing 25 ml of medium. After sterilization at 121° C for 15 min., cultures were inoculated with 1 ml of washed mycelial suspension precultured on the basal medium containing 1 % glucose. Flasks were incubated at 27° C in the dark for 21 days, the mycelial mats separated by filtration with suction through glass filter paper, and toluene (1 ml/l) was added to the filtrate to prevent contamination. The filtrate was stored at 4° C until used.

The following carbon sources and substrates (0.5 % w/v) were used:

Monosaccharides and glycosides. — D-glucose, D-cellobiose, salicin (NBC); p-nitrophenyl-α-D-glucoside, p-nitrophenyl-β-D-glucoside, p-nitrophenyl-α-D-mannoside, p-nitrophenyl-β-D-xyloside (Koch-light); p-nitrophenyl-α-D-galactoside, p-nitrophenyl-β-D-galactoside (NBC).

Polysaccharides. — Avicel cellulose (FMC); sodium carboxymethylcellulose (Fisher, purified with a degree of substitution 0.65 to 0.85); polygalacturonic acid, yeast mannan, galactomannan from guar gum (Sigma); arabinogalactan (Pfaltz and Bauer); xylan (NBC and from bigtooth aspen (*Populus grandidentata* Michx.)), glucomannan (donated by R. Scott, FPL); holocellulose from bigtooth aspen and hemlock (*Tsuga heterophylla* (Raf.) Sarg.); hemicelluloses A and B from bigtooth aspen and hemlock (M. A. Jermyn and F. A. Isherwood, 1956). Molar ratios of glucose : xylose : mannose : galactose in the hemi-

cellulosic fractions were: hemlock A, 8 : 42 : 18 : 15; hemlock B, 16 : 28 : 50 : 6; aspen A, 1 : 95 : 2 : 1; aspen B, 4 : 92 : 3 : 1.

W o o d s. — Ballmilled Engelmann spruce (*Picea engelmannii* Parry) and ballmilled bigtooth aspen (donated by J. OBST, FPL).

Enzyme catalyzed cleavage of glycosidic bonds in polysaccharides and wood substrates was followed by increase in reducing groups at 40° C using Nelson's modification of the Somogyi method (N. NELSON, 1944). Reaction mixtures contained 1 ml of enzymic solution (= culture filtrate) and 1 ml of 1 % substrate in 0.1 M buffer (sodium acetate buffer, pH 4.0 and 5.0; McIlvaine buffer, pH 6.0). A unit of enzyme activity was defined as the amount needed to liberate reducing power equivalent to 1 μM of glucose/16 hr.

α-D-Galactosidase, β-D-galactosidase, β-D-glucosidase, α-D-glucosidase, α-D-mannosidase, and β-D-xylosidase activities were assayed by determining the liberation of p-nitrophenol from the respective p-nitrophenol substrate (K. M. L. AGRAWAL and O. P. BAHL). Two ml of 0.05 % p-nitrophenyl substrate in 0.1 M acetate buffer (pH 5.0) were mixed with 1 ml of enzyme solution and incubated at 40° C. The reactions were terminated by the addition of 1 ml of 0.2 M $NaCO_3$. The resulting yellow color was immediately measured at 425 nm with a spectrophotometer. A unit of enzyme activity was defined as the amount that will liberate 1 μM of p-nitrophenol/hr.

β-D-Glucosidase (cellobiase) was also determined using cellobiose and salicin as substrates. One ml of a 1 % solution of the appropriate substrate in 0.1 M acetate buffer (pH 5.0) was mixed with 1 ml of enzyme solution and incubated at 40° C. Enzymatic cleavage of salicin was followed by increase in reducing groups. Enzyme cleavage of cellobiose was followed by increase in glucose determined by the Glucostat Reagent (Worthington). A unit of enzyme activity was defined as the amount needed to release reducing power equivalent to 1 μM of glucose/hr.

The effect of temperature on glycosidase activity in enzyme solutions was determined by measuring activity at 23°, 40°, 50°, 60°, and 70° C. Temperature stability was determined by holding enzyme solutions at 23°, 40°, 50°, 60°, and 70° C for 1 hr. and assaying glycosidase activity. The effect of pH on glycosidase activity was determined by measuring enzyme activity in various pH's ranging from 2 - 9. The buffers were the following: McIlvaine, pH 2 to 6, 0.05 M; phosphate, pH 7, 0.05 M; and tris-HCl, pH 8 to 9, 0.05 M. The effect of pH on stability was measured by incubating 1 ml of enzyme solution with 1 ml of the buffers for 24 hr. at 4° C, and glycosidase activities determined as described.

Reaction products released during enzymatic degradation were identified by descending paper chromatography. Substrate in 0.1 M acetate buffer, pH 5.0, was incubated with the enzyme solution for varying periods. Ethyl acetate-acetic acid-water (6 : 3 : 3, v/v) and isopropanol-water (4 : 1, v/v) were used as developing solvents. Reaction products were visualized with aniline spray reagent (I. SMITH, 1960).

3. Results

The filtrates of the white-rot fungus *Coriolus versicolor* and the brown-rot fungus *Poria placenta*, cultured with ballmilled aspen and spruce, contained a mixture of extracellular enzymes capable of degrading hemicellulosic polymers and hemicellulosic fractions of a hardwood and softwood (Table 1). Carbohydrolase activities of both fungi were optimal at or near pH 5, and therefore only these results are reported.

Table 1. Cell wall degrading carbohydrate enzymes produced on ballmilled spruce and aspen by *Poria placenta* and *Coriolus versicolor*.

Substrate	Enzyme activity a)							
	P. placenta				*C. versicolor*			
	7 days		21 days		7 days		21 days	
	Aspen	Spruce	Aspen	Spruce	Aspen	Spruce	Aspen	Spruce
Arabinogalactan (larch) ..	0.0	0.0	0.0	0.0	1.2	1.3	1.0	0.5
Galactomannan (guar gum)	2.0	8.8	12.0	6.7	3.5	6.5	8.5	5.7
Glucomannan (pine)	0.7	2.5	4.4	2.8	1.7	2.3	2.8	2.2
Mannan (yeast)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Polygalacturonic acid	6.6	7.2	21.4	21.8	2.0	8.3	5.2	13.9
Xylan (aspen)	9.5	5.1	14.1	15.0	14.6	14.0	32.6	22.9
Xylan (NBC)	10.5	6.5	18.3	17.0	16.3	16.3	37.4	26.6
Hemicellulose A (aspen) ..	6.3	6.3	14.4	10.0	11.3	14.0	32.0	24.6
Hemicellulose A (hemlock)	8.4	12.3	21.8	25.6	15.7	29.0	49.9	50.0
Hemicellulose B (aspen) ..	4.7	3.7	12.7	13.3	8.3	6.0	15.4	9.5
Hemicellulose B (hemlock)	7.7	7.0	15.4	17.5	10.0	10.0	18.4	15.4
Holocellulose (aspen)	0.0	0.0	0.0	0.0	0.8	0.8	1.2	0.7
Holocellulose (hemlock) ..	0.0	0.0	0.0	0.0	0.9	0.9	0.5	0.8
Ballmilled aspen	0.5	1.3	2.7	1.8	2.7	5.0	5.7	0.5
Ballmilled spruce	1.8	3.3	3.5	4.0	3.8	8.3	3.1	2.0

a) A unit of enzyme activity is the amount of enzyme activity which will liberate reducing power equivalent to 1 μM of glucose/16 hr. at 40° C.

The initial products of xylan, glucomannan, and galactomannan hydrolysis were largely oligosaccharides with little free hexose or pentose. With increasing hydrolysis, considerable amounts of monosaccharides appeared. The early appearance of oligosaccharides is characteristic of random cleavage, suggesting that *P. placenta* and *C. versicolor* secrete endohemicellulases. Random cleavage of hemicellulosic materials is commonly reported (G. Keilich, P Bailey, and W. Liese, 1970; H. Sorensen, 1952; P. P. Williams, 1960).

We felt that the spectrum of hemicellulose-degrading enzymes formed by the fungi on aspen substrate might differ from that on spruce. Inability to produce a key hemicellulose-degrading enzyme in a wood,

or production in only small amounts, could be a factor in host preference. However, filtrates of the fungi cultured on spruce contained the same type of enzymatic activities as those cultured on aspen. Although amounts of activity produced on spruce generally differed from those on aspen, differences usually were neither great nor consistent. Furthermore, the filtrates of *C. versicolor* from spruce (slowly degraded by *C. versicolor*) generally had as great or greater activity toward the enzyme substrates as filtrates of *P. placenta* from spruce (rapidly degraded by *P. placenta*).

Neither fungus produced a mannanase capable of hydrolyzing α-mannan from yeast. *C. versicolor* produced hydrolases capable of degrading the remaining substrates. *P. placenta* did not produce enzymes capable of hydrolyzing holocellulose or arabinogalactan.

Filtrates of the brown-rot and white-rot cultures from ballmilled aspen and spruce were more active against commercial xylan and aspen xylan than against glucomannan and galactomannan. Filtrates of *P. placenta* differed only slightly from *C. versicolor* in activity on the mannans. The filtrates of *C. versicolor* were more active toward xylan than those of *P. placenta*. However, *C. versicolor* filtrates did not preferentially degrade the hemicellulosic fractions of aspen even though this fungus produces substantially greater decay in aspen than in hemlock (71 % and 18 %, respectively, in 12 wk). In fact, filtrates of *C. versicolor* were more active toward the hemicellulosic fractions of hemlock than those of aspen. Hemicellulose A of hemlock, which contains a higher proportion of xylan than hemicellulose B (see Methods), was more susceptible to breakdown by both fungi. Filtrates from *P. placenta*, which decays aspen slightly faster than hemlock (70 % and 55 %, respectively, in 12 wk), also were more active toward hemicellulosic fractions of hemlock than aspen.

The brown-rot fungus and white-rot fungus also produced the same types of glycoside-degrading enzymes on spruce as on aspen (Table 2). β-D-Glucosidase and β- and α-D-galactosidases were produced in greatest amounts by *C. versicolor*, α- and β-D-galactosidase in greatest amounts by *P. placenta*. The remaining glycosidases were produced in relatively low amounts; α-D-mannoside was not degraded by either fungus.

P. placenta and *C. versicolor*, however, did differ in the amounts of glycoside-degrading enzyme activities produced on spruce as opposed to aspen. Spruce always induced greater amounts of glycosidase activity by *P. placenta* than aspen did; occasionally the differences were quite large. Aspen, on the other hand, generally induced greater glycosidase activity by *C. versicolor* than spruce did.

Table 2. Glycosidases produced on ballmilled spruce and aspen by *Poria placenta* and *Coriolus versicolor*.

Substrate	Enzyme	Glycosidase activity [a] x 10^2							
		P. placenta				*C. versicolor*			
		7 days		21 days		7 days		21 days	
		Aspen	Spruce	Aspen	Spruce	Aspen	Spruce	Aspen	Spruce
D-Cellobiose	Cellobiase β-D-glucosidase	1.4	6.9	4.6	8.0	21.4	13.5	42.0	14.4
Salicin	β-D-glucosidase	4.0	11.3	7.0	9.8	14.5	8.6	54.0	8.4
β-D-glucoside [b]	β-D-glucosidase	2.3	8.1	3.6	8.2	85.0	65.0	83.1	55.0
α-D-galactoside [b]	α-D-galactosidase	70.4	190.2	120.3	126.2	85.0	65.0	176.0	78.2
α-D-glucoside [b]	α-D-glucosidase	2.0	3.6	3.1	6.7	1.8	2.0	2.4	2.4
β-D-galactoside [b]	β-D-galactosidase	33.3	60.1	44.2	73.0	39.1	39.1	44.2	105.0
β-D-xyloside [b]	β-D-xylosidase	5.2	11.1	8.1	10.1	3.4	4.2	4.0	2.4

a) A unit of enzyme activity is the amount which will release 1 μM of p-nitrophenol or reducing power equivalent to 1 μM of glucose/hr. at 40° C.
b) p-nitrophenyl-glycoside.

The glycosidases of *P. placenta* and *C. versicolor* all exhibited optimal activity at pH 5.0. The glycosidases of *P. placenta*, however, differed from *C. versicolor* in other physical properties, suggesting that glycosidases of *P. placenta* differ structurally from those of *C. versicolor*. Glycosidases of *P. placenta* were stable from pH 2 - 7, whereas glycosidases of *C. versicolor* were stable from pH 4 - 9. The tolerance of the glycosidases of the brown-rot fungus to very acid pH's and those of the white-rotter to alkaline pH's agrees with that found for their cellulases and also coincides with brown-rotted wood usually being more acidic than white-rotted wood.

The optimal temperature for the β-D-glycosidases of *C. versicolor* was 50° C, while the α-D-glycosidases had optimum from 50° - 70° C. Optimal temperatures for β-D-galactosidase, β-D-glucosidase, and β-D-xylosidase of *P. placenta* were 50° C, 60° C, and 60° - 70° C, respectively; optimal temperature of α-D-glycosidases was 50° - 60° C. All of the glycosidases of *C. versicolor* were stable between 23° - 50° C except β-D-xylosidase (23° - 40° C). Likewise, all of the glycosidases of *P. placenta* had the same temperature stability (23° - 60° C), except for β-D-xylosidase (23° - 70° C).

Although there are exceptions, cellulase and mannanase are generally inducible enzymes, while xylanase seems to be largely constitutive (K.-E. ERIKSSON, 1974; K.-E. ERIKSSON and W. RZEDOWSKI, 1969; N. J. KING, 1966; H. LYR, 1960). To study the induction of xylanase, mannanase, CMCase, and glycosidase, the fungi were grown in liquid media which contained either cellulose, sodium carboxymethylcellulose (CMC), galactomannan, xylan, or cellobiose. To estimate enzymatic activities under noninductive conditions, fungi were grown in a medium containing 0.5 % glucose. Enzymatic activities in culture filtrates were determined after 21 days.

Highest yields of mannanase, xylanase, and CMCase from *P. placenta* were obtained with galactomannan as the carbon source (Table 3). The yields of mannanase, xylanase, and CMCase from *P. placenta* were very low with cellulose, but this is probably due to the very poor growth of *P. placenta* on this medium. Mannanase of *P. placenta* appears to be inducible because very little activity was detected in cultures with glucose as the carbon source. As reported previously (T. L. HIGHLEY, 1973), CMCase of *P. placenta* was found to be constitutive. Xylanase of *P. placenta* is apparently semiconstitutive because enzymatic activity is relatively high with glucose as the carbon source, but enzyme levels can be increased inductively.

Highest yields of the same three enzymes from *C. versicolor* were obtained with cellulose or CMC as the carbon source; galactomannan was also a good inducer. Production of these enzymes by *C. versicolor*

Table 3. Effect of carbon source on mannanase, xylanase, and CMCase production by *Poria placenta* and *Coriolus versicolor*.

Carbon source	Enzyme activity [a] in filtrates of					
	P. placenta			*C. versicolor*		
	Mannanase	Xylanase	CMCase	Mannanase	Xylanase	CMCase
Xylan	6.2	5.1	6.5	9.5	9.3	5.3
Glucose	1.9	4.7	6.1	2.2	1.2	1.0
Cellulose (avicel)	0.5	0.2	4.3	12.2	16.8	19.3
Galactomannan	21.1	11.5	9.3	12.2	13.7	9.0
Cellobiose	16.8	6.6	8.9	3.3	0.8	0.8
CMC	17.2	4.4	8.5	13.5	14.6	14.3

a) A unit of enzyme activity is the amount which will liberate reducing power equivalent to 1 µM of glucose/16 hr. at 40° C. Substrate for mannanase, galactomannan; xylanase, xylan (NBC); CMCase, sodium carboxymethylcellulose.

was very low with glucose or cellobiose as the carbon source; xylan was a relatively poor inducer. K.-E. Eriksson and E. W. Goodell (1974) working with the white-rotter *Polyporus adustus,* also found cellulose and mannan to be good inducers of mannanase, xylanase, and CMCase, while xylan and glucose were poor. Thus mannanase, xylanase, and CMCase appear to be inducible for these two white-rotters.

As with the hemicellulases, galactomannan was the best inducer of glycosidase activity in cultures of the brown-rotter *P. placenta* (Table 4). Cellulose was a poor inducer of glycosidase activity, but, as pointed out before, this can probably be attributed to the poor growth of *P. placenta* in the medium with cellulose. Except for α-D-galactosidase activity, cellulose or CMC were the best inducers of glycosidase activities in cultures of *C. versicolor.* α-D-Galactosidase activity was greatest with galactomannan, perhaps due to induction by α-1-6-galactosidic bonds in galactomannan. Cellobiose was a poor inducer of the glycosidases of *C. versicolor,* but a relatively good inducer for *P. placenta.*

4. Discussion

Differences in the ability of soft-rot, white-rot, and brown-rot fungi to utilize hemicelluloses in softwoods and hardwoods has been suggested as a significant factor for the preference of soft- and white-rot fungi for hardwoods and brown-rot fungi for softwoods (G. Keilich, et al., 1970; M. Takahashi and K. Nishimoto, 1973). Rapid growth and consumption of xylose and xylan by the soft-rotter *Chaetomium globosum* have been associated with the greater susceptibility of hardwoods to soft-rot (M. Takahashi and K. Nishimoto, 1973). However, this reasoning may be faulty because they also noted equality of growth on mannose and xylose. Growth on mannan substrates was not determined.

G. Keilich et al. (1970), relate preferential utilization of glucomannan, the main hemicellulose of softwoods, by a brown-rot fungus, and preferential utilization of xylan, the main hemicellulose of hardwoods, by a soft-rotter to the predominant occurrence of brown-rotters and soft-rotters on softwoods and hardwoods, respectively.

In this study, ballmilled spruce induced types and quantities of polysaccharide-degrading enzymes by *P. placenta* and *C. versicolor* similar to those induced by the ballmilled aspen. Further, culture filtrates of *C. versicolor* and *P. placenta* were more active against hemicellulosic fractions of hemlock than against those of aspen. Thus the very little difference between the polysaccharide-degrading enzymes produced by *C. versicolor* and *P. placenta* cultured on ballmilled spruce and aspen could not explain their host preferences .

Table 4. Effect of carbon source on glycosidase production by *Poria placenta* and *Coriolus versicolor*.

Substrate	Enzyme	Glycosidase activities[a] × 10^2 produced on different carbon sources											
		P. placenta						*C. versicolor*					
		1	2	3	4	5	6	1	2	3	4	5	6
Cellobiose	Cellobiase												
	β-D-glucosidase	15.8	8.7	0.5	29.4	14.0	11.4	12.0	5.7	15.5	8.4	2.0	27.2
Salicin	β-D-glucosidase	15.1	10.6	0.0	47.0	13.3	16.7	10.0	7.3	35.2	13.2	2.2	53.3
β-D-glucoside[b]	β-D-glucosidase	22.8	10.3	0.0	90.0	9.0	22.2	91.9	31.4	150.2	62.5	6.0	179.3
α-D-galactoside[b]	α-D-galactosidase	144.0	92.0	6.0	222.0	190.5	156.0	150.6	34.1	170.0	203.8	0.0	156.0
α-D-glucoside[b]	α-D-glucosidase	14.5	4.0	0.0	16.9	1.7	6.6	5.4	1.0	1.0	2.0	0.0	4.7
β-D-galactoside[b]	β-D-galactosidase	99.0	48.0	0.0	159.0	31.2	41.7	129.8	19.4	34.7	81.4	2.4	131.9
β-D-xyloside[b]	β-D-xylosidase	11.1	1.3	1.9	24.0	2.4	3.9	5.6	0.0	4.2	2.0	0.0	14.4

a) A unit of enzyme activity is the amount which will release 1 μM of p-nitrophenol or reducing power equivalent to 1 μM of glucose/hr. at 40° C. 1-xylan, 2-glucose, 3-avicel, 4-galactomannan, 5-cellobiose, 6-CMC. — b) p-nitrophenyl-glycoside.

The activity of glycoside-degrading enzymes produced on spruce was different from that on aspen; greater activities were produced by *P. placenta* on spruce and generally greater activities by *C. versicolor* on aspen. This finding is difficult to relate to host preferences of the fungi, but one wonders if this relationship is true of other brown- and white-rot fungi. Greater production of glycosidases in wood would result in enhanced utilization of cellulose and hemicellulose degradation products, and perhaps in increased decay rates.

The inability of filtrates from *P. placenta* to break down arabinogalactan and its relatively poor degradation by *C. versicolor* are rather surprising, because it is considered to be a key carbohydrate in the establishment of decay fungi in larch (R. W. KENNEDY, 1958). Culture filtrates of *Coniophora cerebella* weakly degraded larch arabinogalactan too (N. J. KING, 1966). That neither fungus can produce enzymes degrading α-mannan from yeast or p-nitrophenyl-mannoside is not unexpected because these chemical linkages are unreported in wood. E. T. REESE and Y. SHUBATA (1965) also found that mannanases from several nonwood-rotting fungi did not hydrolyze α-mannan. However, wood-decay fungi must vary in their capacity to produce enzymes able to cleave α-D-mannosidic linkages, because in another study (Z. ZOUCHOVA, J. KOCOUREK, and V. MUSILEK, 1929) the filtrates of *Trametes sanguinea, Phellinus abietis,* and *Pholioto anrisella* produced enzymes able to hydrolyze α-D-mannosidic linkages of mannan and of 4-nitrophenyl-α-D-mannopyranoside.

The carbohydrolase activities reported here are based on measurements of the extracellular enzymes released by the fungi into their environment. Thus the values probably do not represent total enzyme activities because it is well-known that carbohydrolases can be substrate-absorbed or mycelial-bound. Further insight into the behavior of these fungi might be obtained by study of enzyme release from substrate and mycelium.

Considerable differences are reported in the specificity of glycosidases toward various glycosides (K. M. AGRAWAL and O. P. BAHL, 1968; M. A. JERMYN, 1952; A. J. LUSIS and R. R. BECKER, 1973). However, the glycosidases appear to be specific for the glycopyranosyl group and anomeric configuration of the glycosidic bonds (K. M. AGRAWAL and O. P. BAHL, 1968). For example, α-D-galactosidase and α-D-mannosidase do not show any β-D-glycosidase activity. Likewise, β-D-galactosidase and β-D-glucosidase do not have any α-D-glycosidase activity. They are inactive on macromolecules such as cellulose and glucomannan. The glycosidases were not separated in this study, but from their different physical and inductive properties one might infer the activities to be by

a number of different enzymes. Separating the glycosidases of *P. placenta* and *C. versicolor* and determining their specificities would reveal the number of enzymes necessary during decay to break down the various glycosidic linkages.

Induction of mannanase, xylanase, CMCase, and glycosidases by the white-rot and brown-rot fungi differed on the selected carbon sources. The induction studies provide further evidence that differences in the hemicelluloses of hardwoods and softwoods are most likely unimportant in the preference of white-rotters for softwoods. Cellulose appeared to induce all the necessary enzymes in abundant quantities for the white-rot fungus *C. versicolor* to break down the carbohydrate cell wall components of both hardwoods and softwoods. Mannan was a better inducer of xylanase, CMCase, and mannanase than xylan. Thus, differences in the cellulases, hemicellulases, and glycosidases produced by white-rot fungi on softwoods and hardwoods do not appear to be important in the slower degradative capacities of white-rot fungi on softwoods.

Because the white-rot fungi utilize lignin besides the carbohydrates in wood, differences in the types and amounts of lignin between softwoods and hardwoods may be governing factors in the preference for hardwoods by white-rotters (C. A. Peterson and E. B. Cowling, 1964).

While cellulose was a good substrate for growth and extracellular carbohydrolase production of the white-rotter, it was poor for the brown-rotter. Thus the presence of other carbohydrates in wood apparently plays a more important role in decay of wood by brown-rot fungi than by white-rot fungi. Notable in this study was the abundant induction of the polysaccharidases and glycosidases by galactomannan in cultures of *P. placenta*. However, because galatomannan differs structurally from the mannan in wood, it is difficult to identify the role of mannan in brown-rot decay from these results.

Chemical analysis of brown-rotted coniferous wood showed that glucomannan is removed faster than cellulose, which suggests that further degradation and removal of depolymerized cellulose may depend on prior removal of this major hemicellulose component (T. K. Kirk and T. L. Highley, 1973). H. Lyr (1969) supports the contention that the hemicelluloses are removed before the crystalline cores of cellulosic microfibrils are digested. He finds that hemicellulasic activities of several wood-destroying fungi reached a maximum rapidly and before cellulase activity reached a maximum. Thus, a brown-rot fungus may not infect its host successfully unless the immediate environment is favorable for the production of hemicellulose-degrading enzymes.

Furthermore, circumstantial evidence suggests that the high mannan content of softwoods could enhance the production of cell wall-degrading carbohydrate enzymes, thus enabling the brown-rotters to compete better than white-rotters in the softwood substrate in nature. Other brown-rot fungi and mannans should be studied to identify the function of mannan in the production of cell wall-degrading enzymes by these fungi.

5. Summary

The hemicellulose-degrading enzymes of a brown-rot and a white-rot fungus were compared for differences that could be responsible for fungal host preferences. A very small difference in the hemicellulose-degrading enzymes produced by the white-rotter *Coriolus versicolor* and the brown-rotter *Poria placenta*, cultured on ballmilled aspen and spruce, coincided with the preference of white-rotters for hardwoods and brown-rotters for softwoods. Cellulose apparently induces all the necessary enzymes in abundant quantities for *C. versicolor* to break down the carbohydrate cell wall component of both hardwoods and softwoods. Cellulose was a poor inducer of carbohydrolases of *P. placenta*, while mannan was the best inducer of these enzymes.

Zusammenfassung

Hemicellulasen von Weiß- und Braunfäulepilzen in Beziehung zur Wirtsbevorzugung

Die Hemicellulose-abbauenden Enzyme eines Braun- und eines Weißfäulepilzes wurden hinsichtlich der Unterschiede verglichen, die für die Wirtsbevorzugung der Pilze ausschlaggebend sein können. Ein sehr kleiner Unterschied zwischen den Hemicellulose-abbauenden Enzymen des Weißfäulepilzes *Coriolus versicolor* und des Braunfäulepilzes *Poria placenta*, die auf gemahlener Pappel und Fichte gezüchtet waren, entsprach einer Bevorzugung der Weißfäulepilze für Laubhölzer und der Braunfäulepilze für Nadelhölzer. Cellulose veranlaßt offensichtlich, daß *C. versicolor* alle notwendigen Enzyme in ausreichender Menge zum Abbau sowohl der Kohlenhydrat-Zellwandbestandteile von Laubhölzern als auch von Nadelhölzern bildet. Die Carbohydrolasenbildung von *P. placenta* wird jedoch durch Cellulose kaum angeregt. In Kulturen des Braunfäulepilzes veranlaßte Mannan die Bildung dieser Enzyme am besten.

Résumé

Les hémicellulases de champignons de pourriture blanche et brune en rapport avec les préférences de l'hôte

Les enzymes dégradant les hémicelluloses d'un champignon de pourriture blanche et de pourriture brune ont été comparés pour les différences pouvant être responsables des préférences de l'hôte fongique. Une très petite différence entre les enzymes dégradant les hémicelluloses produits par le champignon de pourriture blanche *Coriolus versicolor* et de pourriture brune *Poria placenta* cultivés sur poudre de peuplier et de sapin, coincidait avec la pré-

férence des champignons de pourriture blanche pour les feuillus et de pourriture brune pour les conifères. La cellulose produisait apparemment tous les enzymes nécessaires en quantité abondante pour que le *C. versicolor* dégrade le composé carbohydrate de la membrane cellulaire à la fois des feuillus et des conifères. La cellulose était un faible inducteur des carbohydrolases de *P. placenta* tandis que le mannane était de meilleur inducteur de ces enzymes dans les cultures de champignons de pourriture brune.

Resumen

Hemicelulasas de hongos causantes de las pudriciones blanca y parda en relación a la preferencia por huéspedes

Se procedió a comparar las enzimas degradantes de la hemicelulosa de dos hongos causantes de la pudrición parda y blanca, respectivamente, con el fin de determinar las diferencias que pudieran ser responsables de la preferencia por huéspedes. Una diferencia muy pequeña entre las enzimas degradantes de la hemicelulosa producidas por el hongo de la pudrición blanca, *Coriolus versicolor*, y del de la pudrición parda, *Poria placenta*, cultivados sobre álamo y abetó molidos, coincidió con la preferencia de los hongos de la pudrición blanca por la madera de fronda, y de los causantes de la pudrición parda por la de coníferas. Aparentemente, la celulosa causa en el *C. versicolor* la creación de todas las enzimas necesarias, y en cantidad suficiente, para la degradación de los componentes hidrato-carbónicos de las paredes celulares, tanto de maderas de fronda como de coníferas. La formación de carbohidrolasas de *P. placenta* apenas se vio provocada por la celulosa, mientras que en cultivos de hongos causantes de la pudrición parda el Mannan fue el que mejor originaba la formación de estas enzimas.

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